Application Note 2016/02

Crocodile ELISA miniWorkstation
Crocodile Control Software

Automation of the Demeditec Cortisol free in Saliva ELISA

Introduction

Cortisol is a steroid hormone released from the adrenal cortex in response to the hormone ACTH (produced by the pituitary gland). Cortisol is involved in the response to stress and can be used as a biomarker of stress. A cortisol test may be used to help diagnose Cushing syndrome, a condition associated with excess cortisol, or to help diagnose adrenal insufficiency or Addison disease, conditions associated with deficient cortisol. Changed patterns of Cortisol levels have also been observed in connection with abnormal ACTH levels, clinical depression, psychological stress, and various physiological stressors as hypoglycaemia, illness, fever, trauma, surgery, fear, or pain.

Cortisol levels in saliva generally show a high degree of correlation with cortisol in serum, and collection of saliva is much easier and more convenient than collection of blood. The Demeditec Cortisol free in Saliva ELISA is a solid phase Enzyme-Linked ImmunoSorbent Assay (ELISA), based on the principle of competitive binding that allows for the quantification of cortisol in saliva with a high sensitivity.

Materials

- Crocodile ELISA MiniWorkstation (Titertek-Berthold)
- Cortisol free in Saliva ELISA (Ref. DES6611, Demeditec)
- Precision micropipettes or multi-dispensing micropipettes, with suitable disposable tips
- Distilled or deionized water

Methods

All reagents were brought up to room temperature for 30 minutes prior to use. Wash Solution was prepared following the manufacturer's instructions. Standards, controls and samples were pipetted according to the manufacturer's instructions.

The Crocodile ELISA miniWorkstation was programmed with the steps summarized in **Table 1**.

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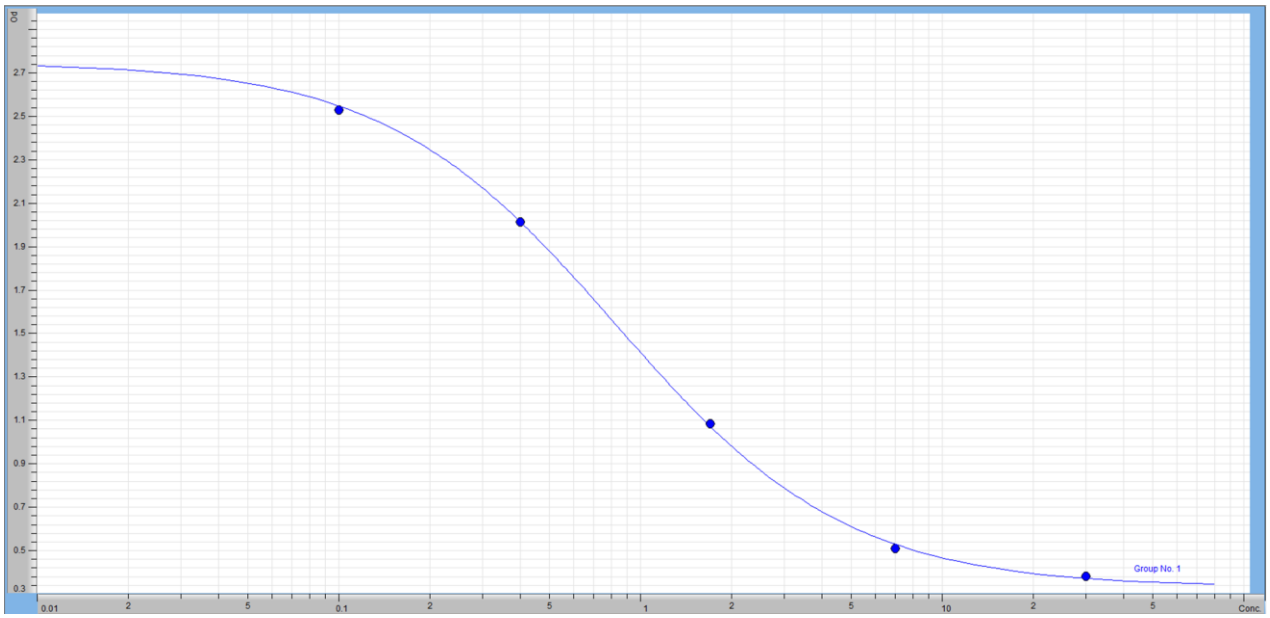
Results

Results are summarized in Figure 1:



Calibrator	Concentration (ng/mL)	OD average (450 nm)
0	0,0	2,762
1	0,1	2,530
2	0,4	2,014
3	1,7	1,084
4	7,0	0,511
5	30,0	0,383

Control	Target value (ng/mL)	Target range (ng/mL)	Calculated concentrations (ng/mL)
1 (Low)	0,29	0,18 - 0,41	0,289
2 (High)	2,17	1,40 - 2,90	1,954

Figure 1. *Top*: standard curve fitted with 4 parameter logistic algorithm; Y-axis linear, X-axis logarithmic. *Bottom left*: Table displaying the results for calibrators. *Bottom right*: Table displaying the results for controls. The calculated concentrations are inside the target range, indicating that the results are valid.

Summary

The results of the test were valid, as the calculated concentrations of both the Low and the High controls were inside the target range. The assay procedure is simple and involves only the addition of controls and samples, while the instrument is processing all necessary dispense, wash, incubation and reading steps. In consequence, the Crocodile ELISA miniWorkstation, in combination with the MikroWin data reduction software, provides a convenient and easy-to-use method to automate the Demeditec Cortisol free in Saliva ELISA.

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Acknowledgements

Data kindly provided by Jon Call from Titertek Instruments.

We wish to thank Demeditec Diagnostics for the supply of reagents.

Table 1. Summary of steps programmed in the Crocodile Control Software

#	Step name	Description and parameters
1	**Conjugate priming**	**Dispensing** Volume: 1000 µL, Inlet: 1, Method: Priming
2	**Conjugate distribution**	**Dispensing** Volume: 50 µL, Inlet: 1, Method: Standard
3	**Mixing**	**Shaking** For 00:01:00, at Shaker Position, with 1 mm Amplitude at 10 Hz
4	**Conjugate incubation**	**Incubating** Incubator Off, duration 01:00:00
5	**Wash Solution priming**	**Washing** Method: Prime Washer, Wash Solution Inlet: 1, Cycles: 6, Volume: 1000 µL
6	**Wash**	**Washing** Method: Standard, Wash Solution Inlet: 1, Cycles: 4, Volume: 300 µL, Delay: 2 s, Wait: 500 ms, Dispenser Depth: 1300 (Plate Offset: -51), Aspiration Depth: 2725* (Plate Offset: 20), Sweep: 5 mm @ 1 mm/s
7	**Substrate priming**	**Dispensing** Volume: 1000 µL, Inlet: 2, Method: Priming
8	**Substrate distribution**	**Dispensing** Volume: 200 µL, Inlet: 2, Method: Standard
9	**Substrate incubation**	**Incubating** Incubator Off, duration 00:30:00
10	**Stop solution priming**	**Dispensing** Volume: 1000 µL, Inlet: 3, Method: Priming
11	**Stop solution distribution**	**Dispensing** Volume: 50 µL, Inlet: 3, Method: Standard
12	**Measure**	**Reading** Single wavelength, Filter 1: 450 nm

* Aspiration depth may have to be optimized for individual Crocodile instruments